REMARKS

Favorable reconsideration of this application is requested. Applicants appreciate the courtesy shown by Examiners Bertagna and Horlick in discussing this case with Applicants' representatives on January 21, 2010. The discussions of the interview are reflected in the following remarks. A copy of the materials (Technical Explanation) discussed during the interview is attached as well. Claim 12 has been canceled without prejudice or disclaimer. Claims 1-11 and 13-17 are pending.

Information Disclosure Statement

The Examiner has indicated that non-patent literature citations 1, 2 and 8 have not been considered, because their citations do not comply with 37 CFR 1.98(b)(5), which requires a date of publication. Applicants respectfully note that the materials in question come from the invalidation trial proceedings, and thus are not publications. Consideration of the materials is respectfully requested.

Claim Objections

Claim 12 is objected to under 37 CFR 1.75(c), as being of improper dependent form. The objection is rendered moot, as claim 12 has been canceled.

Claim rejections - 35 U.S.C. § 103

Claims 1-7 and 9-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rabbani et al. (EP 0971039) in view of Notomi et al. (Nucleic Acids Research 2000; 28(12): e63) and further in view of Nagamine (Molecular and Cellular Probes (June 2002) 16(3):223-229). Applicants respectfully traverse the rejection.

The rejection contends that when all of the evidence is considered, the totality of the rebuttal evidence of nonobviousness fails to outweigh the evidence of obviousness. Applicants respectfully submit that efficient amplification achieved by limiting the parameters as recited in claims 1 and 9 could not have been expected from Rabbani, Notomi and Nagamine. The disclosures of Notomi and Nagamine are substantially similar for the purposes of the present response, so they will be identified together in the remarks.

The mechanism for forming an intermediate between Rabbani and Notomi/Nagamine is different (the differences are illustrated, for example, on page 5 of the Technical Explanation submitted herewith). In particular, in the intermediate formation reaction of Rabbani, after the first turn-back primer anneals to one of the template strands and a new complementary strand is

extended from the turn-back primer annealing site, the 5' tail-end of the turn-back primer hybridizes to the complementary region on the newly synthesized strand so as to form a turn-back loop. When this occurs, the turn-back primer annealing site becomes available, and a second turn-back primer then anneals to the turn-back primer annealing site. Once the second turn-back primer anneals to the turn-back primer annealing site, extension from the turn-back primer annealing site occurs, and the above-mentioned complementary strand that has formed a loop becomes displaced from the template strand, and this displaced strand forms the single-stranded intermediate. The Rabbani disclosure does not provide significant guidance as to how parameters of the turn-back region might affect the efficiency of forming the intermediate.

On the other hand, in the intermediate formation reaction of Notomi/Nagamine, after the first turn-back primer anneals to one of the strands of the genomic DNA and extension from the turn-back primer annealing site occurs so as to synthesize a new complementary strand, the turn-back loop is formed only after the newly synthesized complementary strand is displaced. This is because an outer primer anneals to an outer primer annealing site before the turn-back loop is formed, and when this occurs, a new strand is immediately synthesized, and the previously synthesized complementary strand becomes displaced. This displaced previously synthesized complementary strand then forms the single-stranded intermediate.

The amplification reaction after formation of the single-stranded intermediate is generally similar between Rabbani and Notomi/Nagamine and Notomi/Nagamine merely teach that efficient amplification can be achieved by varying the size of the loop that is formed during the amplification reaction. That is, after the formation of the single-stranded intermediate, a primer that hybridizes to the end opposite to that of the loop (see page 6 of the Technical Explanation; in this illustration, the 3'-end of the single-stranded intermediate) hybridizes to a 3'-end primer annealing site on the single-stranded intermediate. Once the 3'-end primer anneals to the 3'-end annealing site on the intermediate, a new strand complementary to the single-stranded intermediate is synthesized, and dumb-bell shaped loops are formed on each end of the double-stranded intermediate. A turn-back primer annealing site becomes available on the 3'-end loop of the newly made strand complementary to the single-stranded intermediate, and a turn-back primer anneals to this turn-back primer annealing site. It is the size of this 3'-end loop of the newly made strand complementary to the single-stranded intermediate that Notomi/Nagamine teach that can be varied to above 40 bases to achieve better amplification efficiencies. Thus,

Notomi/Nagamine's teaching of varying the loop size is not related to the loop that is formed during the intermediate formation reaction of Rabbani.

The conditions recited in claims 1 and 9 are directed to the self-annealing intermediate formation reaction. In particular, the condition - $1 \leqq (X-Y)/X \leqq 0.75$ (hereinafter, (X-Y)/X referred to as "formula (I)") of claims 1 and 9 relates to the single strand proportion of the target DNA for the annealing region of the second turn-back primer on the 3'-end in the intermediate formation reaction. The condition $30 \leqq X+Y \leqq 50$ (hereinafter, X+Y referred to as "formula (II)") of claims 1 and 9 relates to the length of the loop that is formed in the intermediate formation reaction.

The rejection contends that Notomi teaches that the size of the loop formed between the FIP or BIP primer and the primer extension product, which corresponds to the recited Y value, is critical to the efficiency of the amplification method, and that a loop of 40 bases or longer gave the best results. As indicated above, the loop described by Notomi is the loop that is formed during the amplification reaction, and even if this loop corresponds to the recited Y value, Notomi indicates that Y should be more than 40. On the other hand, claims 1 and 9 are directed to the self-annealing intermediate formation reaction, and requires Y to be less than 40. Y being less than 40 can be deduced from the fact that claims 1 and 9 require X to be between 10 and 30 and X+Y to be between 30 and 50.

Furthermore, according to the conditions - $1 \leqq (X-Y)/X \leqq 0.75$ and $30 \leqq X+Y \leqq 50$ as required by claims 1 and 9, the expression $6 \leqq Y \leqq 33$ can be derived. Specifically, the condition - $1 \leqq (X-Y)/X \leqq 0.75$ can be converted sequentially as follows:

$$-X \leqq (X - Y) \leqq 0.75X$$

$$-2X \leqq -Y \leqq -0.25X$$

$$0.25X \leqq Y \leqq 2X$$

$$0.5Y \leqq X \leqq 4Y.$$

The condition $30 \leqq X+Y \leqq 50$ can then be applied to the thus-obtained $0.5Y \leqq X \leqq 4Y$ equation as follows:

When X+Y = 30, then

$$30/5 \leqq Y \leqq 30/1.5$$

Therefore, $6 \leqq Y \leqq 20$ (A)

When X+Y = 50, then

$50/5 \leqq Y \leqq 50/1.5$

Therefore, $10 \leqq Y \leqq 33$ (because Y is an integer) (B)

Accordingly, based on the expressions (A) and (B), $6 \leqq Y \leqq 33$ can be derived.

When primers having a formula (I) larger than 0.75 are used, the second turn-back primer cannot anneal to the target after the loop formation in the self-annealing intermediate formation reaction. When primers having a formula (I) less than -1 are used, hybridization of the 5' tail-end of the turn-back primer to the complementary region on the newly synthesized strand so as to form a loop in the intermediate formation reaction becomes difficult. On the other hand, when both formulas (I) and (II) are satisfied, enhanced amplification can be achieved. This is demonstrated in the data shown in Tables 1 to 3 in the Technical Explanation.

Table 1 (Tables 1-1, 1-2 and 1-3) summarizes the data from the present specification. Table 2 (Tables 2-1 and 2-2) summarizes the data from the Declaration that was filed July 15, 2009. Table 3 relates to Rabbani's primers. The data in Table 3 will be submitted in a second Rule §1.132 Declaration. The template used in Table 3 is the same as in Table 2 (sY160 STS marker).

Table 3 shows data for primers that are believed to reflect those of Rabbanis (X=20 and Y=0) and primers that satisfy the conditions as required by claims 1 and 9. As is clear from this table, when the primers that best reflect those of Rabbanis are used, amplification was achieved only after 120 minutes. On the other hand, when primers that satisfy the conditions as required by claims 1 and 9 were used, the targeted amplification product was obtained in a reaction time as short as 90 minutes.

The data provided in Tables 1-3 are results obtained using three different target materials (sY160, sY153 and M13) and using a variety of conditions within the range of formula (I) and (II). All of the data in Tables 1-3 are plotted in the graph shown on page 18 of the Technical Explanation. The scope of the claims is illustrated by the shaded area. As is clear from the experimental data and this graph, when formulas (I) and (II) are anywhere outside this scope as defined in claims 1 and 9, efficient amplification cannot be achieved.

Claims 1 and 9 also recite conditions where there is an intervening sequence between Ac' and B'. The number of bases of the intervening sequence is Y', and the conditions recited in claims 1 and 9 for the presence of the intervening sequence are - $1 \leqq \{(X-(Y-Y')\}/X \leqq 0.75$ and $30 \leqq X+Y+Y' \leqq 50$.

Table 4 of the Technical Explanation shows comparative experiments to demonstrate the effects of primers that satisfy the conditions of - $1 \leqq \{(X-(Y-Y')\}/X \leqq 0.75$ and $30 \leqq X+Y+Y' \leqq 50$ as recited in claims 1 and 9. As shown in this table, when primers that do not satisfy the conditions of - $1 \leqq \{(X-(Y-Y')\}/X \leqq 0.75$ and $30 \leqq X+Y+Y' \leqq 50$ as recited in claims 1 and 9 were used, either the desired product was not obtained or amplification was achieved only after 60 minutes (Exp. 1-2(aa) and 1-3(aa) of Table 4) . On the other hand, when primers that satisfy the conditions as required by claims 1 and 9 were used, the targeted amplification product was obtained in a reaction time as short as 40 minutes (Exp. 1-4(aa) to Exp. 1-11(aa) of Table 4).

The data shown in Table 4 is representative of results that are obtained when conditions where there is an intervening sequence between Ac' and B' as recited in claims 1 and 9 are satisfied. This can be understood from the fact that X, Y and Y' are positive integers, X is limited to between 10 and 30, and conditions of - $1 \leqq \{(X-(Y-Y')\}/X \leqq 0.75$ and $30 \leqq X+Y+Y' \leqq 50$ must be satisfied simultaneously. As such, variations in Y' is limited, and Y' being set to two provides a representative example for Y' in general.

In addition, based on the explanation of formula (I) and formula (II), the meaning of the conditions where there is an intervening sequence can be understood by one of ordinary skill in the art without the experimental data.

Firstly, as indicated in the Technical Explanation, formula (I) or (X-Y)/X is related to the proportion of the single strand DNA for the hybridizing region of the second TP. In other words, formula (I) or (X-Y)/X indicates where to position the 3'end of the TP on the loop of the primer elongated strand. Accordingly, in the case where there is an intervening sequence, X+Y' corresponds to X. Here, if the "X" of formula (I) or (X-Y)/X is substituted with "X+Y'", $\{(X-(Y-Y')\}/X$ (hereinafter, formula (I)') is obtained. As such, since the range of formula (I) is $-1 \leqq (X-Y)/X \leqq 0.75$ (which, if rearranged, can be expressed as $0.25 \leqq Y/X \leqq 2$), the range of formula (I)' accordingly will be - $1 \leqq \{(X-(Y-Y')\}/X \leqq 0.75$.

Further, formula (II), as explained in the Technical Explanation, is related to the length of the loop. In the case where there is an intervening sequence Y', Y' also becomes a component of the above-mentioned loop. Accordingly, where there is an intervening sequence, X+Y' corresponds to the X where there is no intervening sequence. Here, if X+Y' is replaced with X, X+Y+Y' (hereinafter, formula (II)') is obtained.

Thus, the scope of formula (I) and formula (II) is the same as that of formula (I)' and formula (II)', respectively. In view of the above discussion, it is clear that the meaning of formulas where there is an intervening sequence, that is, formula (I)' and formula (II)', can be understood by one of ordinary skill in the art without the data provided in Table 4.

From the discussion above, it is clear that the mechanism for the formation of the intermediate is different between Rabbani and Notomi/Nagamine, and that efficient amplification by improving Rabbani's intermediate formation reaction could not have been expected from combining Rabbani with Notomi/Nagamine. The improved effects are clearly demonstrated, for example, in the data shown in Tables 1-4 of the Technical Explanation. Accordingly, claims 1 and 9 and their dependent claims are patentable over the references for at least these reasons.

Claims 8 and 17 are rejected under 35 USC 103(a) as being unpatentable over Rabbani et al. in view of Notomi et al., further in view of Nagamine (Molecular and Cellular Probes (June 2002) 16(3):223-229) and further in view of Kool, E.T. (Current Opinions in Chemical Biology (2000) 4: 602-608). Applicants respectfully traverse the rejection.

Rabbani, Notomi and Nagamine have been distinguished above. Kool does not remedy the deficiencies of Rabbani, Notomi and Nagamine. Therefore, claims 8 and 17 are patentable over the references taken alone or together. Applicants do not concede the correctness of the rejection.

Favorable reconsideration and withdrawal of the rejection are respectfully requested. In view of the foregoing, favorable reconsideration in the form of a notice of allowance is requested. Any questions or concerns regarding this communication can be directed to the attorney-of-record, Douglas P. Mueller, Reg. No. 30,300, at (612) 455.3804.

Respectfully submitted,

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Dated: January 29, 2010

By: _____
James A. Larson
Reg. No.40,443

JAL/DPM/ym

Technical Explanation

US Application 10/532,975

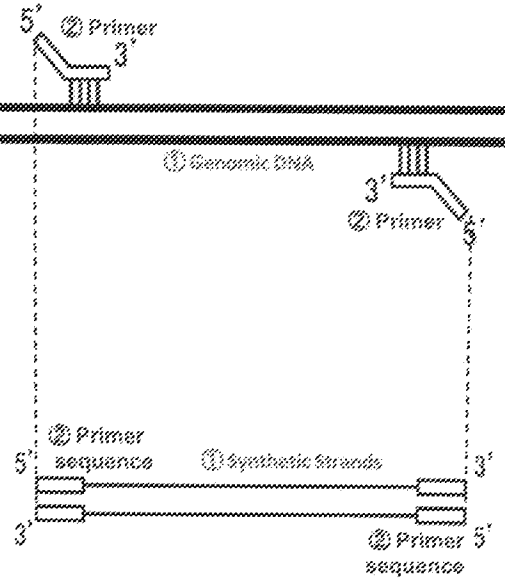
Dr.Yoshihide Hayashizaki

21.01.2010

The gene amplification by use Turn-back primers(TPs)

Starting Materials

① Genomic DNA
② a set of TPs



The intermediate formation reaction

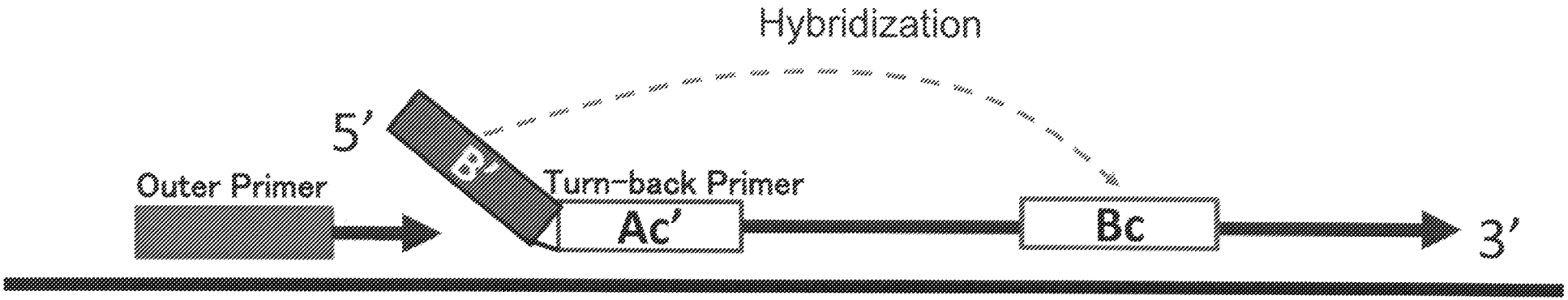
Intermediate

① Synthetic Strands
② Primer sequence

The amplification reaction

Amplification products

Turn-back Primer (TP) & Outer Primer (OP)



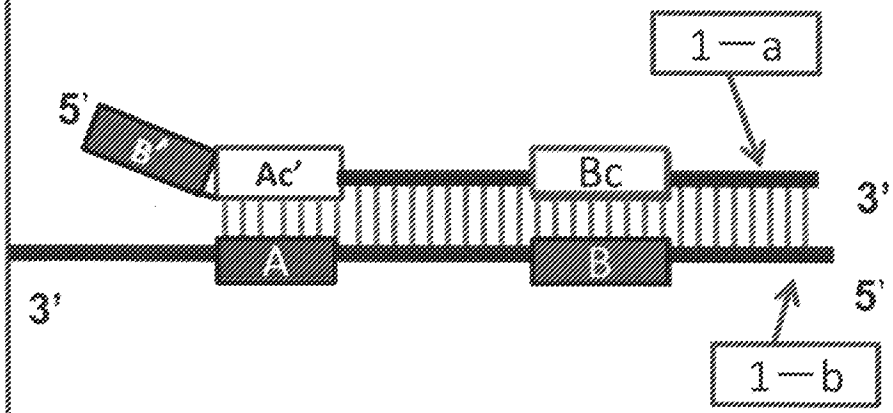
- Notomi uses Outer Primer (OP).
- Rabbani and US Application 10/532,975 does NOT use Outer Primer (OP).

The differences between the intermediate formation reaction and the amplification reaction

Intermediate formation reaction

1—a Elongation strand from TP

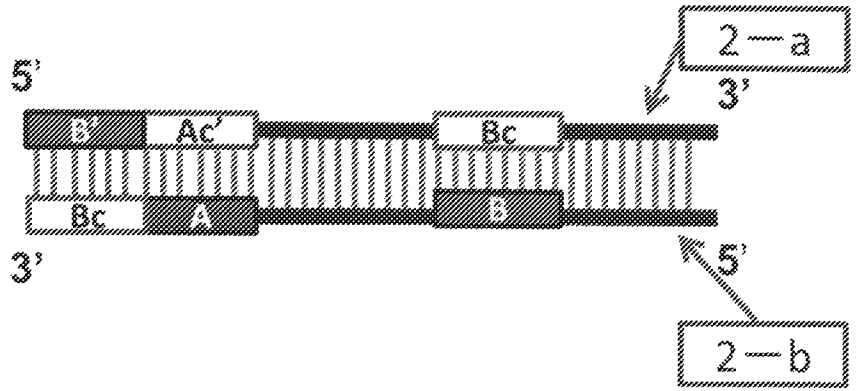
1—b Genomic DNA



Amplification reaction

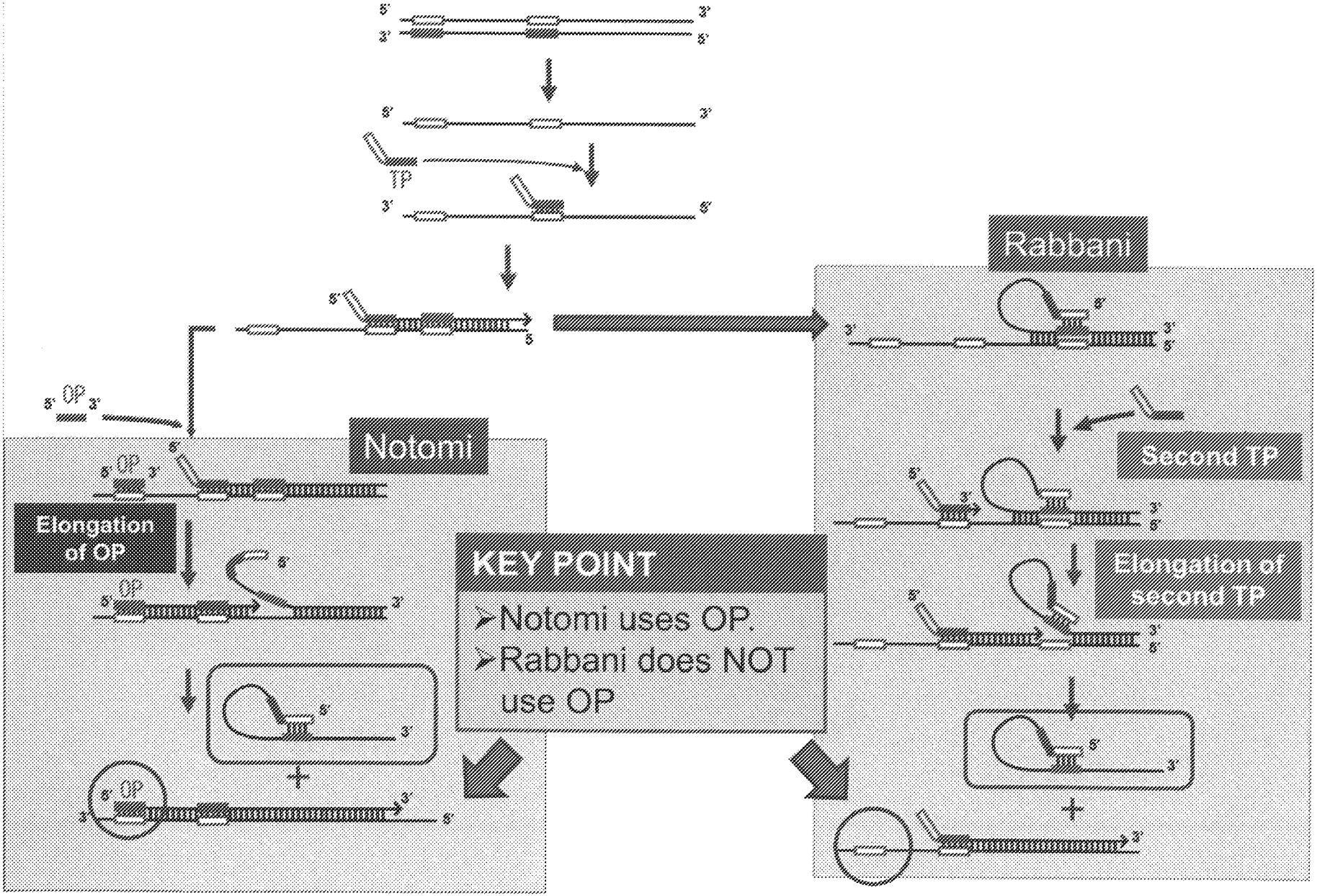
2—a Elongation strand from TP

2—b Complete complementary strand to the 2-a strand



- The template of the intermediate formation reaction is a genomic DNA.
- The template of the amplification reaction is the complete complementary strand to the elongation strand from TP.

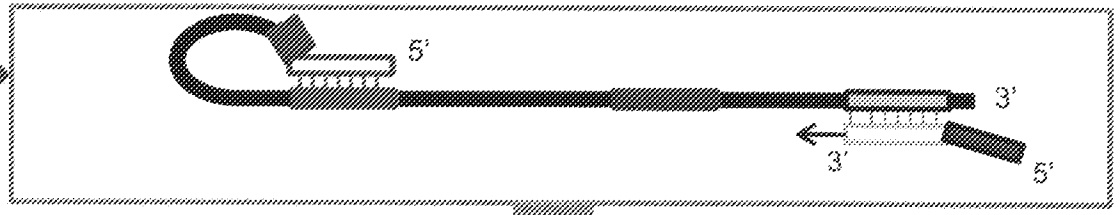
The intermediate formation reaction of “Rabbani and “Notomi”



The amplification reaction

The amplification reaction is similar between"Rabbani"and "Notomi".

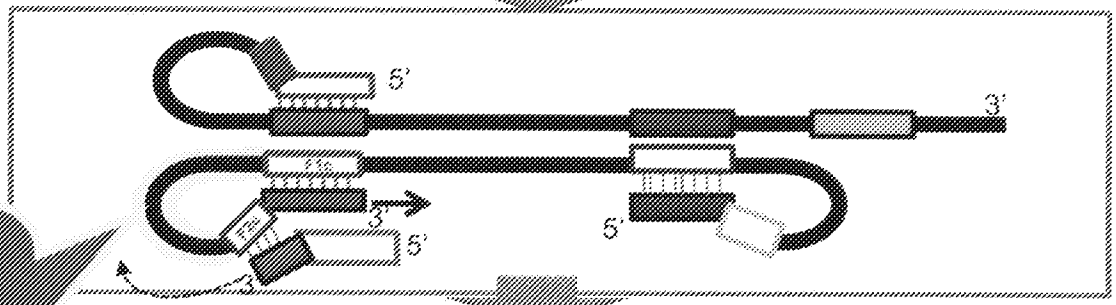
Intermediate



US Application 10/532,975

$30 \leqq X+Y \leqq 50$

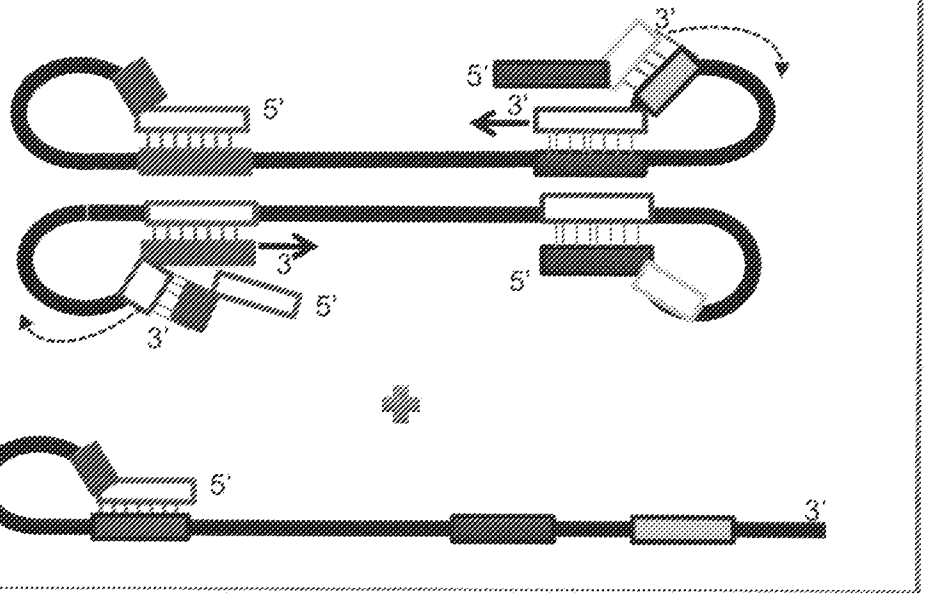
$10 \leqq X \leqq 30 \Rightarrow Y \leqq 40$



Notomi teaches the loop length condition.

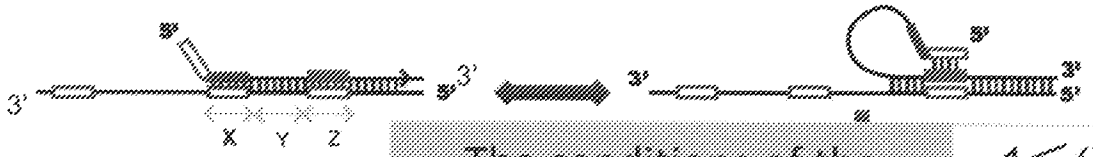
We examined the effect of various sizes of loop between F2c (B2c) and F1c (B1c) on amplification efficiencies and found that a loop of 40 bases or longer gave the best results (data not shown).

Nucleic Acids Research, Vol.28, No.12, e63, 2000



This teaching is Not the TP's turn-back condition but the second TP hybridizing condition to the intermediate's loop.

Aim of and Conditions for efficient gene amplification

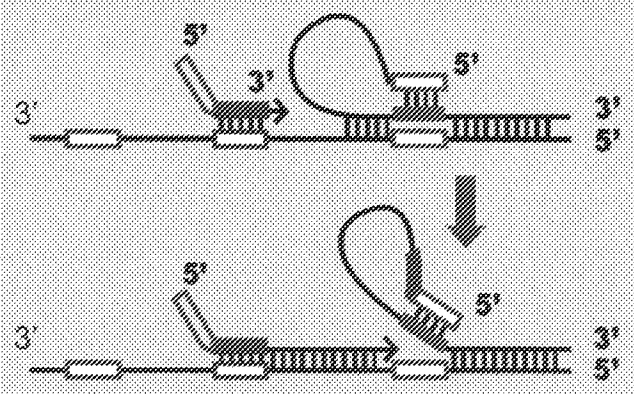


The conditions of the present invention

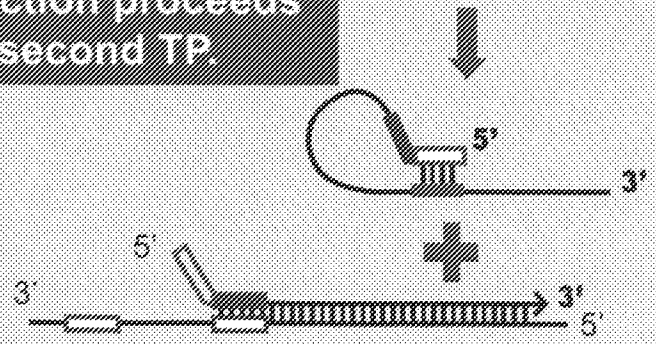
$-1 \leqq (X-Y)/X \leqq 0.75$ (I)

$30 \leqq X+Y \leqq 50$ (II)

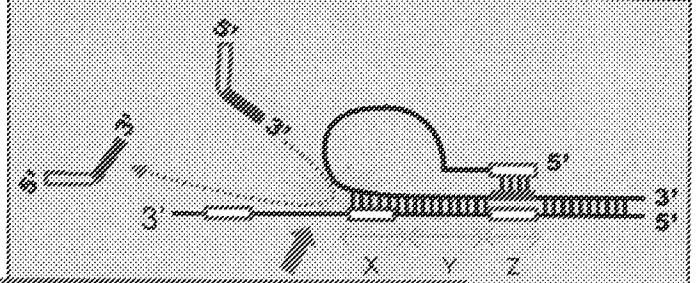
Satisfy



The reaction proceeds by the second TP.



Not Satisfy



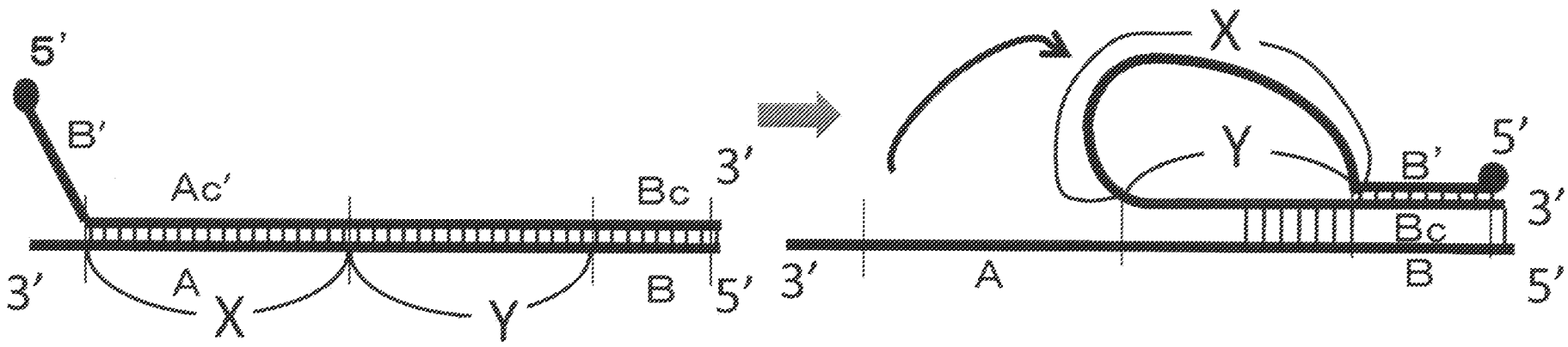
The second TP can NOT hybridize to the template.

The conditions do not allow the second TP to hybridize to the template.

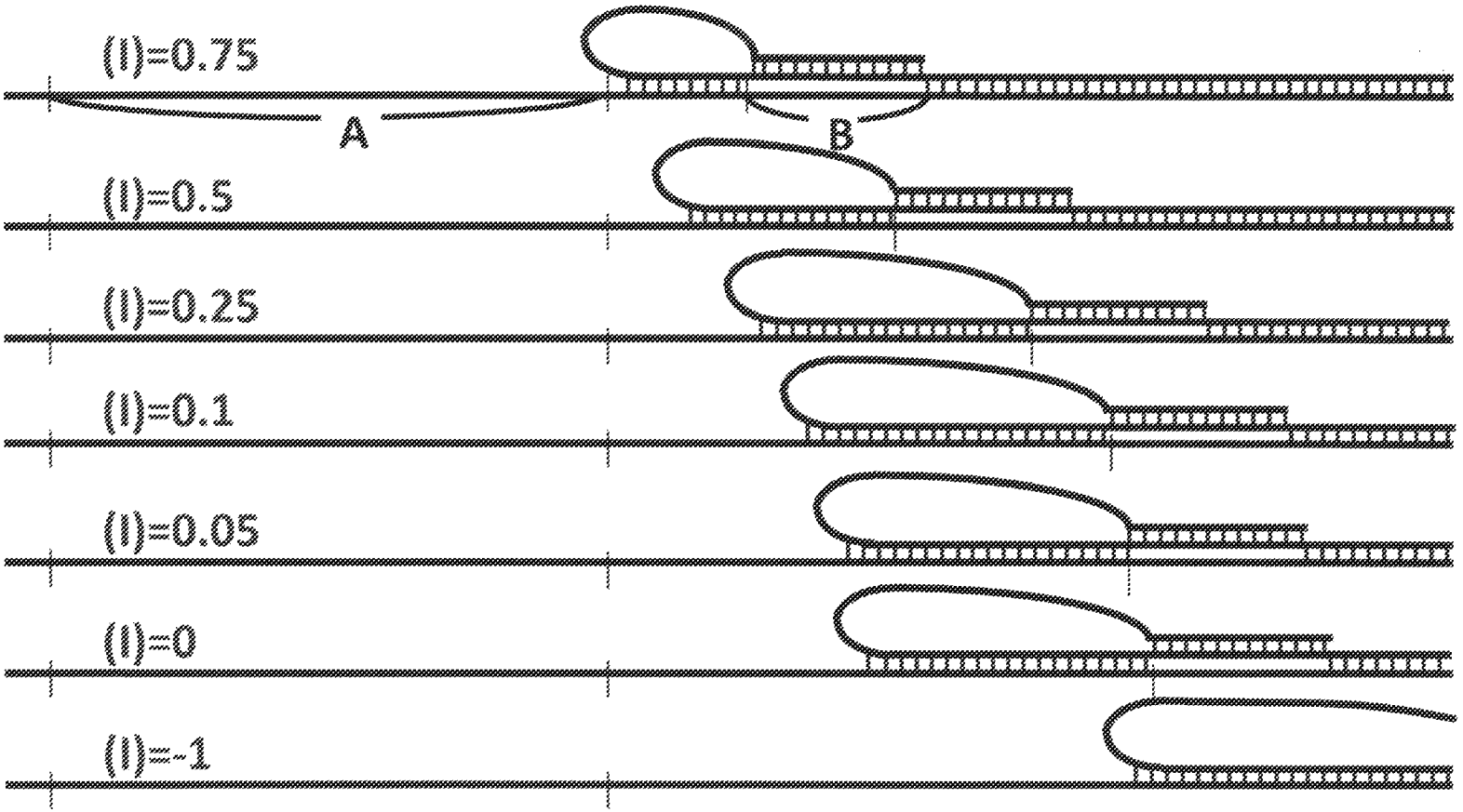
STOP

The significance of formulas (I) and (II)

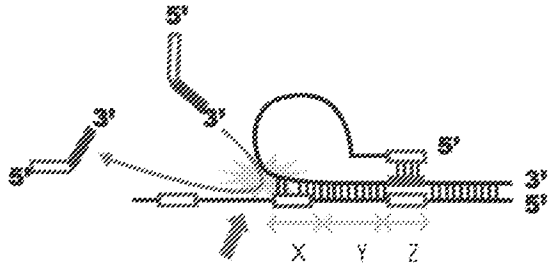
(I) : (X−Y) ／X	(II) : X+Y
The proportion of the single strand DNA for the hybridizing region of the second TP	The length of the loop



The formula(I) is a condition for making sequence (A) into a single strand.

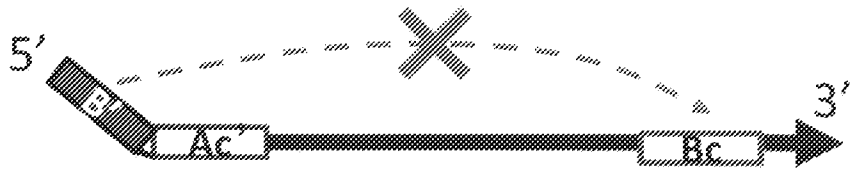


(I)>0.75



The second TP can NOT hybridize to the template.

(I)<-1



The hybridization of B' to Bc is difficult.

Tables (Data from Specification and Declarations)

1. Table 1 to 4 show the empirical data from specification and declarations.
   Table 1 shows the data from examples and comparative examples in specification.
   Table 2 shows the data from the declaration concerning SY160(already filed).
   Table 3 shows the data from the declaration concerning Rabbani's primer.
   Table 4 shows the data from the declaration concerning the intervening sequence Y'.
2. Example Primer sets (satisfy the formula (I) and (II) of the present invention) show enhanced amplification efficiency compared with comparative example primer sets (not satisfy the formula (I) and/or (II) of the present invention) .
3. Efficient amplification achieved by limiting the parameters as recited in claim 1 could not have been expected from Rabbani, Notomi and Nagamine.

Table1-1 (Data from Specification)(1)

Target:SY153(Exp.1)

Primer Set	Primer	Amplification time	Formula	X and Y	(X-Y)/X Formula(Ⅰ)	X+Y Formula(Ⅱ)
Exp.1-1	SY153L	(Non-specific)	Ⅰ:No Ⅱ:No	X=20, Y=--	--	--
	SY153R			X=20, Y=--	--	--
Exp.1-2	SY153LP13-0	60	Ⅰ:No Ⅱ:No	X=20, Y=0	1	20
	SY153RP13-0			X=20, Y=0	1	20
Exp.1-3	SY153LP13-5	60	Ⅰ:Yes Ⅱ:No	X=20, Y=5	0.75	25
	SY153RP13-5			X=20, Y=5	0.75	25
Exp.1-4	SY153LP13-10	40	Ⅰ:Yes Ⅱ:Yes	X=20, Y=10	0.5	30
	SY153RP13-10			X=20, Y=10	0.5	30
Exp.1-5	SY153LP13-15	20	Ⅰ:Yes Ⅱ:Yes	X=20, Y=15	0.25	35
	SY153RP13-15			X=20, Y=15	0.25	35
Exp.1-6	SY153LP10	40	Ⅰ:Yes Ⅱ:Yes	X=20, Y=20	0	40
	SY153RP10			X=20, Y=20	0	40
Exp.1-7	SY153LP13	40	Ⅰ:Yes Ⅱ:Yes	X=20, Y=20	0	40
	SY153RP13			X=20, Y=20	0	40
Exp.1-8	SY153LP16	40	Ⅰ:Yes Ⅱ:Yes	X=20, Y=20	0	40
	SY153RP16			X=20, Y=20	0	40
Exp.1-9	SY153LP22	40	Ⅰ:Yes Ⅱ:Yes	X=20, Y=20	0	40
	SY153RP22			X=20, Y=20	0	40
Exp.1-10	SY153LP25	40	Ⅰ:Yes Ⅱ:Yes	X=20, Y=20	0	40
	SY153RP25			X=20, Y=20	0	40
Exp.1-11	SY153LP28	40	Ⅰ:Yes Ⅱ:Yes	X=20, Y=20	0	40
	SY153RP28			X=20, Y=20	0	40

Table1-2 (Data from Specification)(2)

Target:SY160(Exp.2)

Primer Set	Primer	Amplification time	Formula	X and Y	(X-Y)/X Formula(Ⅰ)	X+Y Formula(Ⅱ)
Exp.2-1	SY160LP13	90	Ⅰ : Yes Ⅱ : Yes	X=20, Y=26	-0.3	46
	SY160RP13			X=20, Y=20	0	40
Exp.2-2	SY160LP16	90	Ⅰ : Yes Ⅱ : Yes	X=20, Y=26	-0.3	46
	SY160RP16			X=20, Y=20	0	40

Table1-3 (Data from Specification)(3)

Target:M13(Exp.3)

Primer Set	Primer	Amplification time	Formula	X and Y	(X-Y)/X Formula(Ⅰ)	X+Y Formula(Ⅱ)
Exp.3-1	M13BIP	(Non-specific amplification)	Ⅰ : No Ⅱ : No	X=24, Y=50	-1.08	74
	M13FIP			X=22, Y=53	-1.41	75
Exp.3-2	M13F2L13-0	90	Ⅰ : No Ⅱ : No	X=24, Y=0	1	24
	M13R2L13-0			X=22, Y=0	1	24
Exp.3-3	M13F2L13-6	60	Ⅰ : Yes Ⅱ : Yes	X=24, Y=6	0.75	30
	M13R2L13-6			X=22, Y=6	0.73	28
Exp.3-4	M13F2L13-12	60	Ⅰ : Yes Ⅱ : Yes	X=24, Y=12	0.50	36
	M13R2L13-12			X=22, Y=12	0.45	34
Exp.3-5	M13F2L13-18	60	Ⅰ : Yes Ⅱ : Yes	X=24, Y=18	0.25	42
	M13R2L13-18			X=22, Y=18	0.18	40
Exp.3-6	M13F2L13	60	Ⅰ : Yes Ⅱ : Yes	X=24, Y=22	0.08	46
	M13R3L13			X=22, Y=22	0	44
Exp.3-7	M13F2L23	60	Ⅰ : Yes Ⅱ : Yes	X=24, Y=22	0.08	46
	M13R3L23			X=22, Y=22	0	44

Table2-1 (Data from Declaration concerning SY160)(1)

1. Declaration according to amplifying the sY160 gene.
2. Comparative Examples are the same to Examples except that they do not satisfy the formula (I) and (II) of the present invention.
3. Therfore, the relationship between the amplification efficiency and the formula (I) and (II) are clear.

Target:SY160(Exp.2)

Primer Set	Primer	Amplification time	Formula	X and Y	(X-Y)/X Formula(Ⅰ)	X+Y Formula(Ⅱ)
Exp.2-1	SY160LP13	90	I : Yes II : Yes	X=20, Y=26	-0.3	46
	SY160RP13			X=20, Y=20	0	40
Exp.2-1(Yes/No)	SY160LP13-1	(Non-specific amplification)	I : Yes II : No	X=20, Y=5	0.75	25
	SY160RP13-1			X=20, Y=9	0.55	29
Exp.2-1(No/Yes)	SY160LP13-2	150	I : No II : Yes	X=15, Y=35	-1.333	50
	SY160RP13-2			X=15, Y=35	-1.333	50
Exp.2-1(No/No)	SY160LP13-3	(Not amplified)	I : No II : No	X=20, Y=56	-1.8	76
	SY160RP13-3			X=20, Y=56	-1.8	76

Table2-2 (Data from Declaration concerning SY160)(2)

Target:SY160(Exp.2)

Primer Set	Primer	Amplification time	Formula	X and Y	(X-Y)/X Formula(Ⅰ)	X+Y Formula(Ⅱ)
Exp.2-2	SY160LP16	90	Ⅰ : Yes Ⅱ : Yes	X=20, Y=26	-0.3	46
	SY160RP16			X=20, Y=20	0	40
Exp.2-2(Yes/No)	SY160LP16-1	(Non-specific amplification)	Ⅰ : Yes Ⅱ : No	X=20, Y=33	-0.65	53
	SY160RP16-1			X=20, Y=9	0.55	29
Exp.2-2(No/Yes)	SY160LP16-2	(Not amplified)	Ⅰ : No Ⅱ : Yes	X=15, Y=35	-1.333	50
	SY160RP16-2			X=15, Y=33	-1.2	48
Exp.2-2(No/No)	SY160LP16-3	150	Ⅰ : No Ⅱ : No	X=20, Y=51	-1.55	71
	SY160RP16-3			X=20, Y=45	-1.25	65

Target:SY160(Exp.2)

Primer Set	Primer	Amplification time	Formula	X and Y	(X-Y)/X Formula(Ⅰ)	X+Y Formula(Ⅱ)
Exp.2-3(Yes/Yes)	SY160 TP-F(16,32)	90	Ⅰ : Yes Ⅱ : Yes	X=16, Y=32	-1	48
	SY160 TP-R(16,32)			X=16, Y=32	-1	48

Table3 (Data from Declaration concerning Rabbani's primer)

Target:SY160(Exp.2)

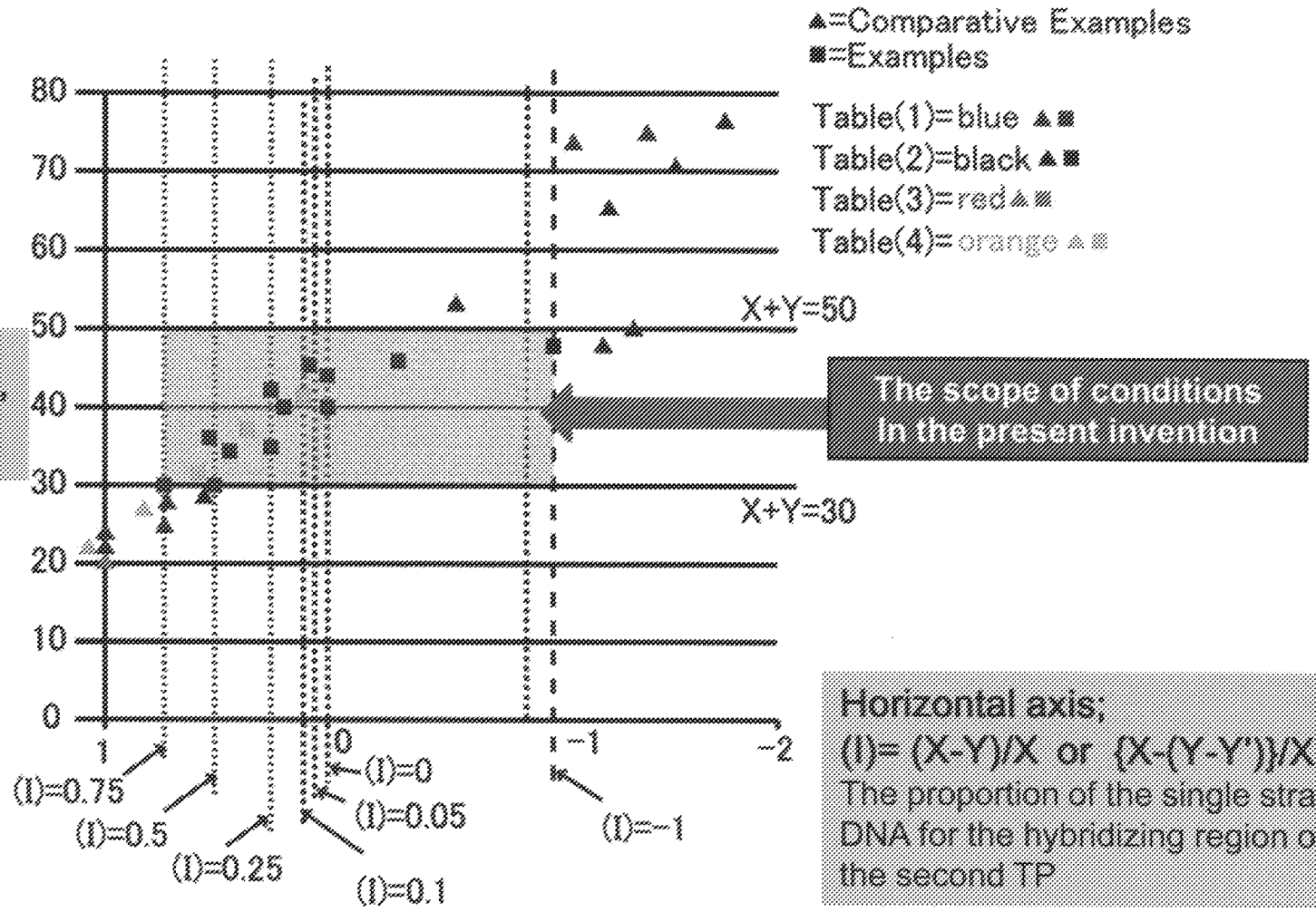
Primer Set	Primer	Amplification time	Formula	X and Y	(X-Y)/X Formula(Ⅰ)	X+Y Formula(Ⅱ)
Exp.2-1	SY160LP13	90	Ⅰ : Yes Ⅱ : Yes	X=20, Y=26	-0.3	46
	SY160RP13			X=20, Y=20	0	40
Exp.2-2	SY160LP16	90	Ⅰ : Yes Ⅱ : Yes	X=20, Y=26	-0.3	46
	SY160RP16			X=20, Y=20	0	40
Exp.2-1(Y=0)	SY160LPY013	120	Ⅰ : No Ⅱ : No	X=20, Y=0	1	20
	SY160RPY013			X=20, Y=0	1	20
Exp.2-2(Y=0)	SY160LPY016	120	Ⅰ : No Ⅱ : No	X=20, Y=0	1	20
	SY160RPY016			X=20, Y=0	1	20

Table4 (Data from Declaration concerning Y')

Target:SY153(Exp.1)

Primer Set	Primer	Amplification time	Formula	X, Y and Y'	(X-(Y-Y'))/X Formula(Ⅰ)	X+Y+Y' Formula(Ⅱ)
Exp.1-2(aa)	SY153LP13-5A2	(Not amplified)	Ⅰ : No Ⅱ : No	X=20, Y=0, Y'=2	1.1	22
	SY153RP13-0A2			X=20, Y=0, Y'=2	1.1	22
Exp.1-3(aa)	SY153LP13-5A2	60	Ⅰ : No Ⅱ : No	X=20, Y=5, Y'=2	0.65	27
	SY153RP13-5A2			X=20, Y=5, Y'=2	0.65	27
Exp.1-4(aa)	SY153LP13-10A2	60	Ⅰ : Yes Ⅱ : Yes	X=20, Y=10, Y'=2	0.4	32
	SY153RP13-10A2			X=20, Y=10, Y'=2	0.4	32
Exp.1-5(aa)	SY153LP13-15A2	60	Ⅰ : Yes Ⅱ : Yes	X=20, Y=15, Y'=2	0.25	37
	SY153RP13-15A2			X=20, Y=15, Y'=2	0.25	37
Exp.1-6(aa)	SY153LP10A2	60	Ⅰ : Yes Ⅱ : Yes	X=20, Y=20, Y'=2	0.1	42
	SY153RP10A2			X=20, Y=20, Y'=2	0.1	42
Exp.1-7(aa)	SY153LP13A2	60	Ⅰ : Yes Ⅱ : Yes	X=20, Y=20, Y'=2	0.1	42
	SY153RP13A2			X=20, Y=20, Y'=2	0.1	42
Exp.1-8(aa)	SY153LP16A2	60	Ⅰ : Yes Ⅱ : Yes	X=20, Y=20, Y'=2	0.1	42
	SY153RP16A2			X=20, Y=20, Y'=2	0.1	42
Exp.1-9(aa)	SY153LP22A2	60	Ⅰ : Yes Ⅱ : Yes	X=20, Y=20, Y'=2	0.1	42
	SY153RP22A2			X=20, Y=20, Y'=2	0.1	42
Exp.1-10(aa)	SY153LP25A2	60	Ⅰ : Yes Ⅱ : Yes	X=20, Y=20, Y'=2	0.1	42
	SY153RP25A2			X=20, Y=20, Y'=2	0.1	42
Exp.1-11(aa)	SY153LP28A2	60	Ⅰ : Yes Ⅱ : Yes	X=20, Y=20, Y'=2	0.1	42
	SY153RP28A2			X=20, Y=20, Y'=2	0.1	42

Plane graph of formulas (I) and (II)



Vertical axis;
(II)=X+Y or X+Y+Y'
The length of the loop

The scope of conditions in the present invention

Horizontal axis;
(I)= (X-Y)/X or {X-(Y-Y')}/X
The proportion of the single strand DNA for the hybridizing region of the second TP

1. Primers according to the specification of the present application and the three declarations.
2. Example Primer sets (satisfy the formula (I) and (II) of the present invention) show enhanced amplification efficiency compared with comparative example primer sets (not satisfy the formula (I) and/or (II) of the present invention) .